Dust and grit matter: abrasives of different size lead to opposing dental microwear textures in experimentally fed sheep (Ovis aries)

Ackermans, Nicole L ; Winkler, Daniela E ; Martin, Louise F ; Kaiser, Thomas M ; Clauss, Marcus ; Hatt, Jean-Michel

Abstract: External abrasives ingested along with the herbivore diet are considered main contributors to dental wear, though how the different sizes and concentrations of these abrasives influence wear remains unclear. Dental microwear texture analysis (DMTA) is an establishedmethod for dietary reconstruction-which describes a tooth's surface topography on a micrometre scale. The method has yielded conflicting results as to the effect of external abrasives. In the present study, a feeding experiment was performed on sheep (Ovis aries) fed seven diets of different abrasiveness. Our aim was to discern the individual effects of size (4, 50 and 130 m) and concentration (0%,4% and 8% of dry matter) of abrasives on dental wear, applying DMTA to four tooth positions. Microwear textures differed between individual teeth, but surprisingly, showed no gradient along the molar tooth row, and the strongest differentiation of experimental groups was achieved when combining data of all maxillary molars. Overall, a pattern of increasing height, volume and complexity of the tooth's microscopic surface appeared with increasing size of dietary abrasives, and when compared with the control, the small abrasive diets showed a polishing effect. The results indicate that the size of dietary abrasives is more important for dental microwear texture traces than their concentration, and that different sizes can have opposing effects on the dietary signal. The latter finding possibly explains conflicting evidence from previous experimental DMTA applications. Further exploration is required to understand whether and how microscopic traces created by abrasives translate quantitatively to tissue loss.

DOI: https://doi.org/10.1242/jeb.220442

RESEARCH ARTICLE

Dust and grit matter: abrasives of different size lead to opposing dental microwear textures in experimentally fed sheep (*Ovis aries*)

Nicole L. Ackermans[1,*], Daniela E. Winkler[2,3], Louise F. Martin[1], Thomas M. Kaiser[3], Marcus Clauss[1] and Jean-Michel Hatt[1]

ABSTRACT

External abrasives ingested along with the herbivore diet are considered main contributors to dental wear, though how the different sizes and concentrations of these abrasives influence wear remains unclear. Dental microwear texture analysis (DMTA) is an established method for dietary reconstruction which describes a tooth's surface topography on a micrometre scale. The method has yielded conflicting results as to the effect of external abrasives. In the present study, a feeding experiment was performed on sheep (*Ovis aries*) fed seven diets of different abrasiveness. Our aim was to discern the individual effects of size (4, 50 and 130 µm) and concentration (0%, 4% and 8% of dry matter) of abrasives on dental wear, applying DMTA to four tooth positions. Microwear textures differed between individual teeth, but surprisingly, showed no gradient along the molar tooth row, and the strongest differentiation of experimental groups was achieved when combining data of all maxillary molars. Overall, a pattern of increasing height, volume and complexity of the tooth's microscopic surface appeared with increasing size of dietary abrasives, and when compared with the control, the small abrasive diets showed a polishing effect. The results indicate that the size of dietary abrasives is more important for dental microwear texture traces than their concentration, and that different sizes can have opposing effects on the dietary signal. The latter finding possibly explains conflicting evidence from previous experimental DMTA applications. Further exploration is required to understand whether and how microscopic traces created by abrasives translate quantitatively to tissue loss.

KEY WORDS: Microtexture, Feeding experiment, Ruminant, Diet, Abrasives, Tooth wear

INTRODUCTION

Tooth wear is an important factor for dietary reconstruction, and herbivores have been well documented to show contrasting dental wear for different diets. A diet of browse is thought to induce more attrition, resulting in teeth with sharp molar cusps (Fortelius and Solounias, 2000), and on a microscopic level, an enamel surface with wear facets dominated by pits and a microtexture with low roughness (Schulz et al., 2013a,b; Winkler et al., 2019a). A diet of grass, in contrast, is thought to include internal and often adherent external abrasives that wear down the tooth material, resulting in blunted molar cusps, scratch-dominated microwear facets, and a microtexture with high roughness, anisotropy and low complexity (Scott, 2012; Schulz et al., 2013a). It is still under debate whether phytoliths – the hard opaline silicates internal to plants – or external abrasives, like dust or grit, are the main cause of tooth wear in herbivores (reviewed in Winkler et al., 2019a). Some believe that external abrasives do not matter (Merceron et al., 2016), though others found that they might (Hoffman et al., 2015). We aimed to use the present experiment with diets that include different concentrations and sizes of abrasives to further contribute to this debate.

Baker et al. (1959) were the first to observe microscopic pitting on sheep teeth caused by phytoliths, in what is thought to be the first application of the 2D microwear technique, before Walker et al. (1978) introduced 2D microwear as a systematic method to deduct herbivore diets. Microwear is used to analyse microscopic wear on a tooth's surface, quantifying small scratches and pits in order to ascertain the diet of a specimen. As the turnover for these surface marks is a matter of days or weeks (Teaford and Oyen, 1989b), the short time frame of this proxy has earned it the moniker of 'the last supper' effect (Grine, 1986). Adaptations of industrial systems have led to less use of 2D microwear in favour of 3D techniques (reviewed in Scott et al., 2006; see fig. 1 of Calandra et al., 2019). Today, the two leading techniques in dental microwear texture analysis (DMTA) are scale-sensitive fractal analysis (SSFA) and 3D surface texture analysis (3DSTA). Both SSFA and 3DSTA quantify the texture of the whole surface as a unit (Clementz, 2012) and use profilometry at sub-micrometre resolution to represent 3D geometry and distribution of topographic features on the tooth's enamel surface (Scott et al., 2006; Schulz et al., 2010). SSFA uses four main parameters [area-scale fractal complexity (Asfc), anisotropy (epLsar), heterogeneity of complexity (HAsfc) and textural fill volume (Tfv); full details are available from Dryad Table S1: https://doi.org/10.5061/dryad.x95x69pdm] to describe various surface features that vary with scale of observation. With SSFA, grazers tend to exhibit high anisotropy and either systematically low (Ungar et al., 2007; Scott, 2012; Merceron et al., 2014) or high (Schulz et al. 2010) complexity values, while browsers show the opposing tendency. These opposing complexity values are most likely due to inter-microscope differences, a technical issue that requires further calibration and collaboration between working groups. 3DSTA characterises wear features using over 40 parameters subdivided into six categories of analysis (direction, furrow, isotropy, ISO 12781, ISO 25175 and motif). Using 3DST analysis, tooth surfaces of grazing animals generally show high surface roughness, high peaks in great quantity, deep dales and a general pattern that is low in variability. Tooth surfaces of browsers, in contrast, generally show flatter surfaces with lower peaks. Microwear and DMTA have been applied to several non-mammalian taxa, including reptiles (Winkler et al., 2019b) and fish (Purnell et al., 2012), but for the

[1]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland. [2]Applied and Analytical Paleontology, Institute for Geosciences, Johannes Gutenberg University Mainz, 55099 Mainz, Germany. [3]Center of Natural History, University of Hamburg, 20146 Hamburg, Germany.

*Author for correspondence (nlackermans@gmail.com)

N.L.A., 0000-0001-8336-1888; D.E.W., 0000-0001-7501-2506; T.M.K., 0000-0002-8154-1751; M.C., 0000-0003-3841-6207; J.-M.H., 0000-0002-7043-7430

Received 16 December 2019; Accepted 9 January 2020

most part these methods have been applied to mammals (Ungar et al., 2007; Schubert et al., 2010; Merceron et al., 2014; Brent Jones and Desantis, 2017; Aiba et al., 2019), including extinct humans (Pérez-Pérez et al., 2003).

Overall, both SSFA and 3DSTA are used to determine diet based on its inherent mechanical properties, though the exact connection between these properties and the wear traces they leave has so far mainly been derived from logical reflections rather than empirical relationships (Kaiser et al., 2016). Consequently, controlled feeding experiments are the only means by which to corroborate the interaction between microtexture formation and dietary properties. Experimental studies have been performed in various mammals such as possums (Kay and Covert, 1983), primates (Teaford and Oyen, 1989a,b; Teaford et al., 2017) including humans (Romero et al., 2012), rabbits (Schulz et al., 2013b), various rodents (Kropacheva et al., 2019; Mihlbachler et al., 2019; Winkler et al., 2019a) and sheep (Hoffman et al., 2015; Merceron et al., 2016; Ramdarshan et al., 2017), and *in vitro* with chewing machines (Hua et al., 2015; Daegling et al., 2016; Karme et al., 2016). Although such a wide array of species have been investigated, the traces recorded by DMTA remain to be fully understood, especially at the species level.

To address in particular the conflicting results in sheep – the model organism for large herbivores, which represents the group on which DMTA is most often applied in palaeontological diet and climate reconstructions – we performed a feeding experiment, using three different sizes of abrasives at two dietary concentrations (resulting in seven diets, including an abrasive-free control diet). Although DMT patterns are expected to establish within days to a week, the diets were fed consistently for a period of 17 months, in order to include analyses other than DMTA that will be investigated at a later stage. Based on previous findings, we expected a pattern of increasing dental microwear with increasing size and concentration of abrasives, as well as a decreasing pattern of wear on the gradient from the maxillary M1 towards the M3, and higher wear on the mandibular m2 in comparison to its maxillary counterpart.

MATERIALS AND METHODS

Samples

The teeth analysed in this study belonged to the specimen collection related to the feeding experiment described in Ackermans et al. (2019) and Ackermans et al. (2020b), performed with the approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment licence no. 10/2016). Forty-eight ewes and one wether (*Ovis aries* Linnaeus 1758, n=49) were fed experimental diets for 17 months in a controlled experimental setting. The animals were divided into seven groups based on mass and fed pelleted diets of varying abrasiveness. Using a low-abrasive lucerne (*Medicago sativa*) based pellet, three sizes (diameter: 4, 50 and 130 µm) and two concentrations (4% and 8%) of quartz abrasives were added to create the different diets, leading to a total of seven diets: a control diet with no added abrasives (C), two diets with small abrasives (4%s and 8%s), two diets with medium abrasives (4%m and 8%m) and two diets with large abrasives (4%l and 8%l). A complete description of the diets is reported in Ackermans et al. (2019). At the end of the experiment the skulls were skeletonised and housed in the mammal collection at the Center of Natural History of the University of Hamburg.

Microwear texture analysis

DMTA of the samples was performed following the standard technique in Schulz et al. (2013a). We applied 46 dental microwear texture (DMT) parameters using the ISO 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness) and SSFA. DMTA parameters were grouped into the following categories for simplification: area (Sda, Sha, mea), complexity (Sdr, nMotif, Asfc), density (Sal, Spd, medf), direction (Std, Str, Tr1R, Tr2R, Tr3R, IsT, epLsar), height (S10z, S5p, S5v, Sa, Sku, Sp, Sq, Ssk, Sv, Sxp, Sz, meh, madf, metf, FLTt, FLTp, FLTq, FLTv), peak sharpness (Spc), plateau size (Smc, Smr), Slope (Sdq) and volume (Sdv, Shv, Vm, Vmp, Vmc, Vv, Vvc, Vvv) (for description, see Dryad Table S1: https://doi.org/10.5061/dryad.x95x69pdm). Excluding the animals that died early during the feeding experiment and those unsuitable for microwear texture analysis due to cracked or chipped cusps, we were left with a sample size of n=37.

For each specimen, four sites on the same facet were analysed following Schulz et al. (2013a), when possible: the posterior facet of the anterior cusp on the second enamel band (from the buccal side) for the right maxillary M1, M2 and M3 (Fig. 1), using the same facet on the fourth enamel band when the previous was damaged or too worn. In cases where both sites were not measurable in a right tooth, the left side was used for all tooth positions. The antagonist facet to the M2 for was selected for each specimen on the mandibular m2: the anterior facet of the posterior cusp on the fourth enamel band (from the buccal side) was selected on the m2 when the ideal facet was measured on the M2 (Fig. 1); however, when the fourth enamel band was measured on the M2, the second enamel band was selected on the m2. In preparation for measurements, the target facets were cleaned with rubbing alcohol and then moulded using putty (Heraeus Kulzer Provil® novo light cd. dental putty). A copper wire was inserted into the mould as a guide to indicate direction, and the moulds were placed into a microtitre plate (Schulz et al., 2013a). The plate was then fixed to the table of the confocal disc scanning microscope (µsurf custom, NanoFocus AG, Oberhausen, Germany). The microscope was equipped with a blue LED (470 nm) and a high-speed progressive-scan digital camera (984×984 pixels), set to a 100× long distance objective (resolution in x,y=0.16 µm, step size in z=0.06 µm). Four scans of 160×160 µm were rendered per facet, taking care not to overlap scanning areas. The data were processed with MountainsMap Premium (v7.4.8803, DigitalSurf, Besançon, France; www.digitalsurf.com). 3D scan images were created using MountainsMap (Fig. 2).

Statistical analysis

Statistical analysis was carried out using R software (v3.3.1; http://www.R-project.org/) with the packages xlsx (https://CRAN.R-project.org/package=xlsx), rJava (https://CRAN.R-project.org/package=rJava), doBy (https://CRAN.R-project.org/package=doBy) and R.utils (https://cran.r-project.org/web/packages/R.utils/index.html). Analysis was performed on the following groupings: all teeth, maxillary teeth (M1, M2 and M3), antagonist group (M2 and m2), and each individual tooth (M1, M2, M3 and m2). Significance was tested using a combination of three statistical tests. As DMT data are generally non-normally distributed, we used the procedure of Wilcox (2012), applying a robust T1-way heteroscedastic Welch–Yuen omnibus test, coupled with a heteroscedastic pairwise Dunnett's T3 test, with significance confirmed using the robust heteroscedastic rank-based test according to Cliff (pairwise comparison with bootstrap), with methodology following that of Calandra et al. (2012) and Schulz et al. (2013a). The significance level was set to 0.05. Boxplots and selected biplots were created for each grouping, with different letters indicating significant differences at P<0.001, and significance indicated with asterisks (*P<0.05, **P<0.01 and ***P<0.001). Principal component analysis (PCA)

Journal of Experimental Biology (2020) 223, jeb220442. doi:10.1242/jeb.220442

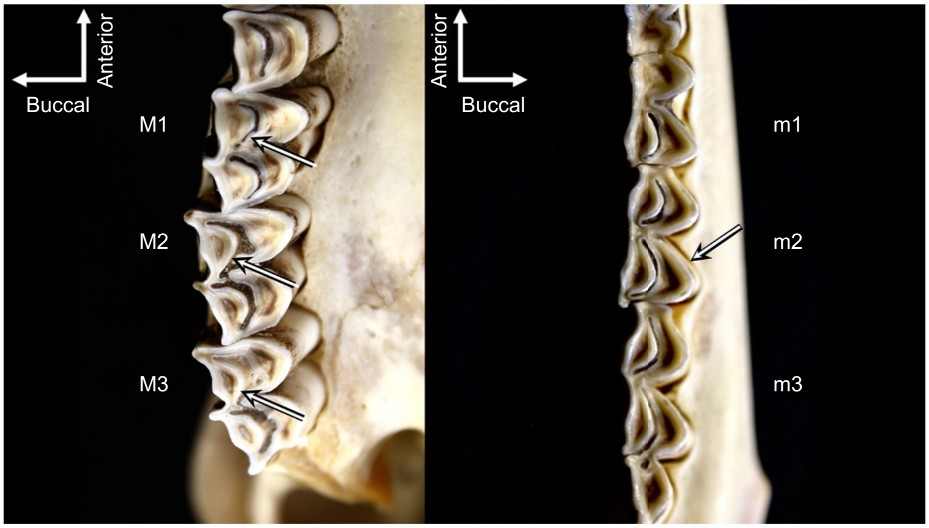

Fig. 1. Wear facet measured using dental microtexture analysis on teeth of sheep (*Ovis aries*). Arrows indicate wear facets where microtexture was sampled and scanned. The left image represents the right maxillary molar row and the right image represents the right mandibular molar row.

was created in R v3.3.1 using the built in function *prcomp* with singular value decomposition (SVD) and ggbiplot (http://github.com/vqv/ggbiplot) for visualisation. Predicting variables were *z*-transformed and the PCA was based on correlations in order to ignore different scale of variables. A Kaiser–Meyer–Olkin measure of sampling adequacy (value >0.5) using the function 'paf' of the R package rela (https://CRAN.R-project.org/package=rela) and Bartlett's test indicate that the formal requirements for conducting a PCA were met by our data. Detailed results for the PCA have been deposited in Dryad (https://doi.org/10.5061/dryad.x95x69pdm). In order to facilitate different statistical approaches to data analysis by other researchers, the original data have also been deposited in Dryad (https://doi.org/10.5061/dryad.x95x69pdm).

RESULTS

Boxplots and descriptive statistics for all individual measurements are available from Dryad (Tables S1–S9 and Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm). Fig. 2 shows sample images of the surface texture of the enamel facet for all teeth from a single individual from each diet group. Visually, texture directionality

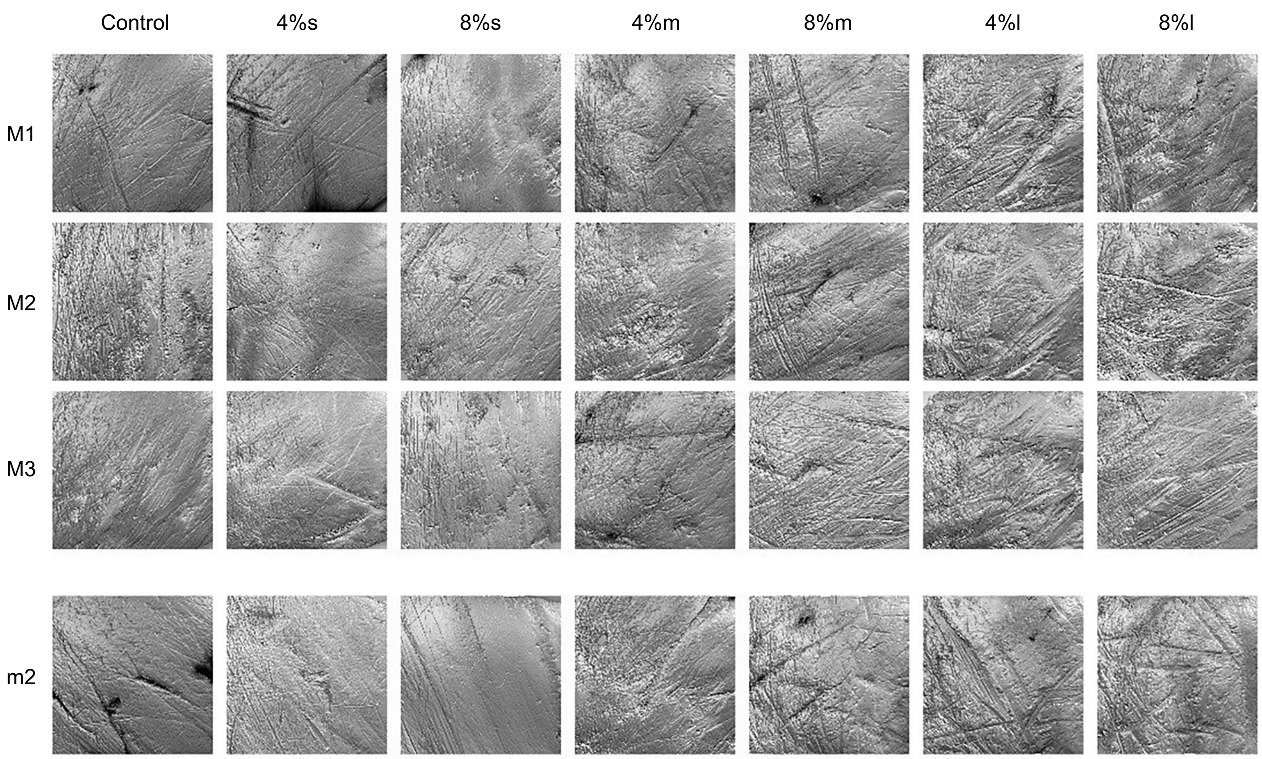

Fig. 2. Microwear texture photosimulations of the enamel tooth surface of experimental sheep fed diets of varying abrasiveness for 17 months. Images were created using MountainsMap software. Specimens imaged here are ZMH10941 (control), ZMH10942 (4%s), ZMH10922 (8%s), ZMH10910 (4%m), ZMH10956 (8%m), ZMH10919 (4%l) and ZMH10913 (8%l), where 4% and 8% represent the concentration of abrasives of small (s, 4 µm), medium (m, 50 µm) and large (l, 130 µm) diameter. The same facet was imaged for the maxillary M1, M2 and M3, and the antagonist facet of M2 was imaged for the mandibular m2. Images were all taken with the same orientation, with anterior to the top and buccal side to the left of the figure; each image represents a 160×160 µm section of a facet.

was more or less consistent between maxillary molars, and showed an opposing directionality on the mandibular molar. A clear polishing effect was visible across teeth for the 8%s diet and to a lesser extent for the 4%s diet, and surface roughness appeared to increase with increasing size of dietary abrasives, with the largest abrasives creating large, deep scratches. Boxplots of select parameters for all maxillary molars combined are provided in Fig. 3.

Biplots and PCA

Plotting complexity against anisotropy (Asfc versus epLsar) showed no clear diet group distinction for all maxillary molars combined (Fig. 4A; see Dryad Fig. S3: https://doi.org/10.5061/dryad.x95x69pdm), though the dietary groups consuming small abrasives separated somewhat from the other groups. Using complexity (Asfc) on its own provided better differentiation between diet groups (Fig. 3). Plotting depth and density of furrows against each other (metf versus medf) for all maxillary molars combined showed a lot of scatter in the control and medium-sized abrasives groups, though both small abrasives groups were well separated by high density and shallow furrows (medf and metf). The large abrasives groups showed the opposite distribution of low medf and high metf (Fig. 4B; see Dryad Fig. S3: https://doi.org/10.5061/dryad.x95x69pdm).

This parameter combination showed a similar resolution for the dietary groups as compared with a PCA with the 12 best separating parameters. On PC1, groups were mainly separated by height and volume parameters, with the large-sized abrasives diets showing larger parameter values. On PC2, the smallest abrasives diets were mainly separated by density of furrows (medf) (Fig. 4C).

Maxillary molar (M1, M2, M3) wear gradient

Overall, there were very few significant inter-tooth differences and there was no significant indication or visual pattern of a gradient along the upper molar row (see Dryad Table S3 and Fig. S2: https://doi.org/10.5061/dryad.x95x69pdm).

Antagonist tooth (M2 and m2) wear patterns

An obvious pattern was seen for directional parameters for the small- and medium-sized abrasives diets when comparing the two second molars (see Dryad Table S4 and Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm). The texture direction (Tr1R) of the mandibular m2 showed wear features predominantly aligned at higher angles (predominantly buccally oriented) and distributed more randomly, while the maxillary M2 showed strong features oriented predominantly at lower angles (Tr1R: 50 deg, Tr2R: 40–60 deg; see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

Diet differences for all teeth

Area parameters showed no clear pattern in relation to diet for combined or individual teeth, though the values in mandibular m2 were slightly lower when compared with those of other teeth (see Dryad Tables S2–S4 and Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

Complexity parameters showed significant differences between diets (see Dryad Table S2: https://doi.org/10.5061/dryad.x95x69pdm). When upper molars were combined, they displayed an increasing trend in complexity (Fig. 3, Asfc), and the same was true for individual teeth, with M1 and m2 showing increasing complexity (Sdr, Asfc) with increasing particle size, and smaller particles showing lower complexity as compared with the control group. However, that pattern was not visible in the M2 and M3, except for the 8%s diet, which showed lower complexity (Sdr, Asfc; see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

Density parameters showed a decreasing density of furrows (medf) with increasing particle size when all maxillary molars were combined (Fig. 3), and when considering individual teeth, the same pattern was seen for all but the M2. There was also a tendency for increasing density of peaks (Spd) with increasing particle size in the M1 and m2 (see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm). In general, the diets with larger particles created fewer features (medf) but also more peaks (Spd).

For direction parameters, when upper molars were combined, they showed some variation but the direction of wear features (Tr1R) was predominantly between 50 and 60 deg. For individual teeth, Tr1R was the same in the M1 and M2 (around 50 deg), and more variable in the M3, while it was much higher in the m2 (80–100 deg). Small abrasive diets showed lower isotropy (IsT) in the M1 and M3, and M3 had higher anisotropy (epLsar) for the larger abrasives (see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

Most height parameters indicated that when combining data for the upper molars, diets with the smallest abrasives of either concentration created lower height and less overall roughness (Fig. 3, metf, Sa), a pattern that was also visible for individual teeth. In the mandibular m2, a tendency of increasing height (meh) and roughness (Sq) with increasing abrasive size appeared; this trend, however, was not as pronounced in the individual maxillary teeth. The most important finding is that depth of furrows (metf) was consistently lowest in the diets with small abrasives and increased with particle size for all teeth. Only in the maxillary M3 did the 4%s diet diverge from the general pattern (see Dryad Tables S2–S4 and Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

Combined data for the molars showed plateau size increasing significantly with size of the abrasives (Fig. 3, Smc), and the same trend was visible for individual teeth (see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

The slope parameter indicated only a slight tendency for increasing slope with increasing particle size in combined data for molars, and this trend was much more pronounced for M1, M2 and m2 (see Dryad Fig. S1, Sdq: https://doi.org/10.5061/dryad.x95x69pdm).

Finally, volume parameters, similar to height parameters, indicated significant increases in topography with size of abrasives for combined molars (Fig. 3, Vmc) as well as individual molars (see Dryad Fig. S1: https://doi.org/10.5061/dryad.x95x69pdm).

DISCUSSION

Application of DMTA to sheep's teeth after they had been fed experimental diets containing abrasives of increasing size and concentration for 17 months revealed no molar gradient between the maxillary M1–M3, and few differences between the M2 and its mandibular antagonist, m2. However, individual tooth differences appeared, and combining all scans provided the best overall dietary distinction. There were opposite effects of external abrasives depending on their size, with diets containing small abrasives creating a polishing effect on the enamel surface, and diets with abrasives of increasing size creating increasing enamel surface roughness. The results thus reconcile conflicting findings of other experiments that did or did not find an effect of external abrasives, using abrasives of different sizes (Hoffman et al., 2015; Merceron et al., 2016).

Individual and grouped tooth differences

Contrary to our expectations, there was no indication of a wear gradient measured by DMTA along the maxillary molar row. When recording DMT or 2D microwear in mammalian herbivores, the

Journal of Experimental Biology (2020) 223, jeb220442. doi:10.1242/jeb.220442

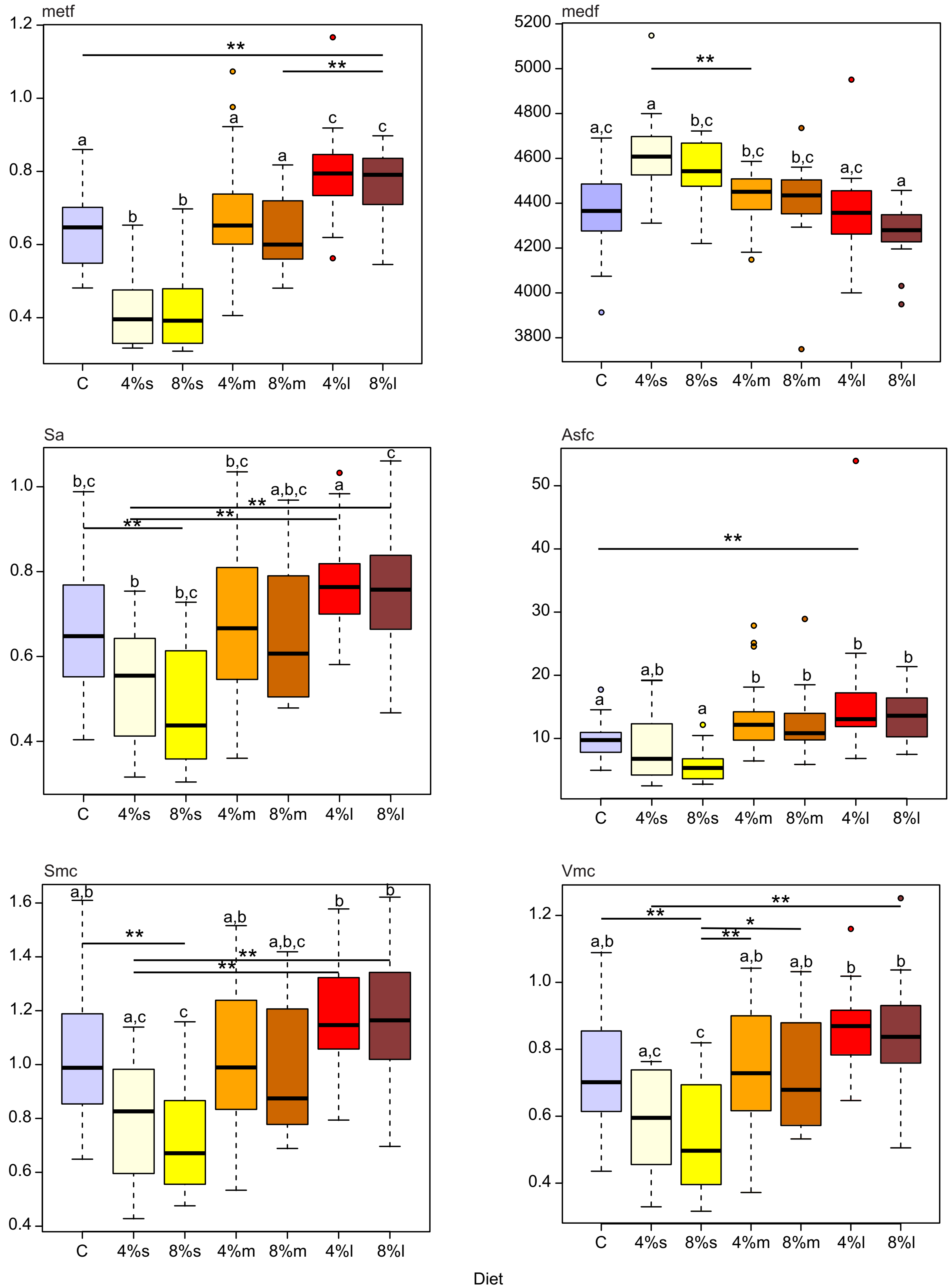

Fig. 3. Boxplots of texture analysis in combined maxillary molars (M1+M2+M3) of sheep (*n*=37) fed diets with varying abrasives for 17 months. Diets are as defined in Fig. 2. Only those parameters showing a significant difference for all three statistical tests are included: metf (µm) and medf (cm cm^{-2}) represent the depth and density of furrows, respectively, Sa (µm) represents mean height and surface roughness, Asfc represents complexity, Smc (µm) represents plateau size and Vmc (µm^3 µm^{-2}) represents volume. Different letters indicate a significant difference of *P*<0.001. Asterisks indicate a significant difference of **P*<0.05 and ***P*<0.01.

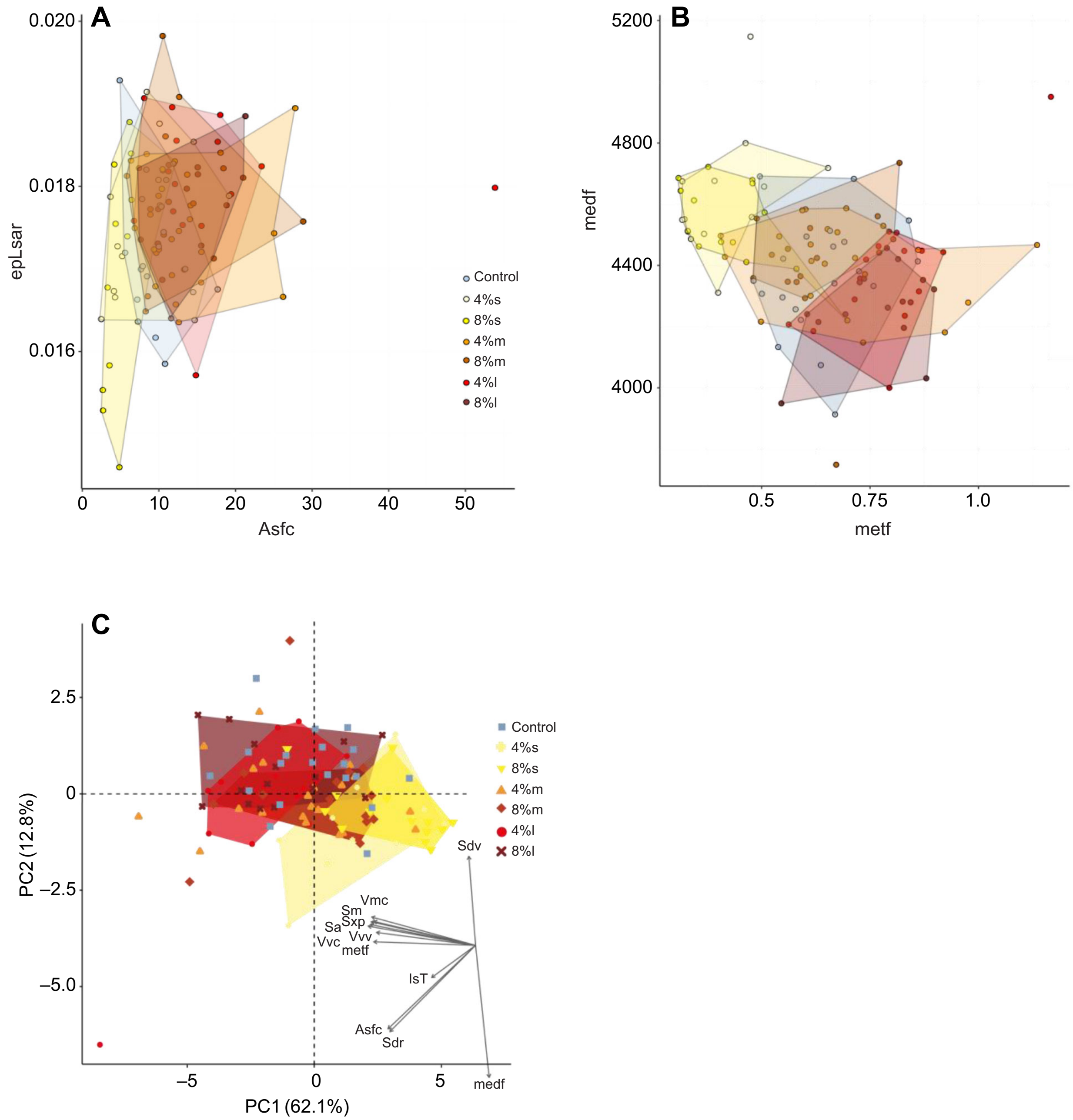

Fig. 4. Biplots and principal component analysis (PCA) of combined maxillary molars (M1+M2+M3) of sheep (*n*=37) fed diets with varying abrasives for 17 months. Diets are as defined in Fig. 2. (A) Biplot of Asfc (complexity) and epLsar (anisotropy). (B) Biplot of medf and metf (mean density and depth of furrows, respectively). (C) PCA of the 12 strongest parameters. Sdv, Vmc, Vvv, Vvc: volume; Sm: plateau size; Sxp, Sa, metf: depth; IsT: direction; Asfc, Sdr: complexity; medf: density. For parameter description, see Dryad Table S1 (https://doi.org/10.5061/dryad.x95x69pdm).

maxillary M2 is most often used as the tooth of reference. This is based on the sequence in which the molars erupt (Monson and Hlusko, 2018). The M1 erupts first and thus is often already heavily worn in adult individuals of a sample population. The M3 erupts last and may, therefore, often not be in full use and representative for the individual's diet. This leaves the M2 as the putatively best molar for a representative result. Correspondingly, we expected the M1 to show more wear on its occlusal surface, the M2 to show intermediate wear and the M3 to show the least distinct wear pattern. Such a wear gradient has been recorded on the macroscopic scale in the literature for many species, including cercopithecine monkeys (Gantt, 1979), ibex (Fandos et al., 1993), giraffes (Clauss et al., 2007), rhinoceroses (Taylor et al., 2013), and between premolars and molars in wild equids (Taylor et al., 2016). Whereas macroscopic wear may be directly related to the age of the tooth, this is not necessarily the case for DMT, which is thought to develop quickly within the course of several days to weeks. The absence of a gradient in our own results therefore need not be considered surprising. In fact, the M2 showed the least discrimination between dietary groups, when the number of significant parameters was taken as a measure.

In other microtexture studies, molar measurements are often combined into a single dataset to maximise sample size, regardless of tooth position (Ungar et al., 2007; Merceron et al., 2010, 2014; Schulz et al., 2013a). Tooth position and gradient effects have been investigated using DMTA in ungulates; and Schulz et al. (2010)

observed a molar gradient for density (Sal) and texture direction (Std) parameters in wildebeest (*Connochaetes gnou*), which they interpreted to be linked to rumination, as well as to the high curvature of the tooth row in wildebeest as compared with zebra. Contrastingly, Ramdarshan et al. (2017) found no such gradient along the tooth row of experimental sheep in a controlled feeding trial.

Mandibulary antagonist teeth were also investigated by Ramdarshan et al. (2017), and when applying SSFA parameters, no differences between maxillary and mandibulary molars were observed. This resembles our own results, as the only significant differences between the M2 and m2 were related to texture direction (Tr1R). These differences in texture direction may be caused by the mortar and pestle function of the maxillary and mandibular antagonist (Kaiser and Fortelius, 2003). Our results showed that the M3 had the highest number of significant parameters, while the M1 showed the best differentiation between groups, and the dietary differentiation for the mandibular m2 was comparable to that of the M1, putting into question the use of the M2 as the standard tooth of reference for microtexture analysis. With individual teeth each showing differing trends in our results, it is important that these differences are reflected in the interpretation, considering that it may be preferable to measure the complete molar tooth row, rather than focusing on the M2, as a single tooth may yield less differentiating power. For differentiating between dietary groups, the best discrimination was achieved by combining all maxillary molars, showing that regardless of tooth position, a higher number of scans across different teeth may be more important for parameter significance in dietary discrimination.

Dietary differences

All parameters considered, a general trend of diets with larger abrasives resulting in larger and more prominent microtexture features was evident for all teeth. Surprisingly, different concentrations of abrasives generally had a negligible effect on the creation of microtexture features. The only exception was the 8%s diet, which often created a much stronger polishing effect than the 4%s diet, as visible in Fig. 2. Unexpectedly, the base pellets used for the control diet created a fair amount of surface roughness. The addition of small abrasives to the pellets allowed the diet to ‘polish away’ that roughness on the whole surface texture, while abrasives of other sizes did not show a polishing effect. A polishing effect may be characteristic of the ingestion of extremely small dust particles and may be relevant for palaeontological dietary reconstruction. For example, it may link a specimen to an open, windswept habitat. The decreasing density of peaks (Spd) with particle size indicates in particular that the abrasion of peaks was caused by smaller particles. This also explains the lower number of significant parameters for the medium-sand diets, as their range of wear often overlapped with that of the control diet (Fig. 3). A similar ‘polishing effect’ has previously been discussed by Sanson et al. (2017), in that a large particle can cause a microscopic scratch, and smaller particles can then wear away the edges of this scratch, resulting in the appearance of less microscopic surface roughness. In an experiment testing the effect of external abrasives on sheep using DMTA, Merceron et al. (2016) saw no effect from the addition of less than 0.8% of dust (diameter >100 µm, comparable in size to our medium-sand diet, though in lower concentration) to a browse- or graze-based diet for 70 days. Therefore, even though small particles create a polishing effect, and larger particles create increasing surface roughness, abrasives in the range of 100 µm diameter may not create a distinctive microwear texture on small ruminant teeth, a result emulated by the medium-sized abrasives in the present study.

In our results, both the height and volume parameters showed the best dietary discrimination across all teeth (e.g. Sa, metf, Vmc), and height, volume and complexity parameters increased relatively consistently with the size of abrasives for all teeth. medf and metf (mean density and depth of furrows, respectively) were particularly strong parameters in the present study, which also was the case for guinea pigs and lepidosaurs (Winkler et al., 2019a,b); additionally, Schulz et al. (2010) noted high medf values specifically in ruminants compared with non-ruminants fed similar diets. In the present study, Asfc (complexity) on its own showed strong discrimination between dietary groups, with large abrasives resulting in high complexity (Fig. 3). As expected, direction parameters rarely differed between teeth or diet groups, as they reflect chewing direction, which should be consistent within a species. Seemingly, a more abrasive diet (i.e. with larger abrasives) leads to more wear on all scales, with more overall tissue loss and deeper microscopic furrows.

Importantly, whether deeper furrows, higher roughness or a polished surface translate to more or less tissue loss is still uncertain. Microscopic wear may in some cases be the result of tissue deformation rather than tissue loss, where a scratch represents merely a plastic deformation of the enamel (Lucas et al., 2013). In particular, DMTA cannot quantify the frequency with which microwear traces occur on a tooth; in theory, deeper furrows, while detectable, might be created at a much lower frequency than small furrows, and hence do not represent a difference in the rate of tissue loss. DMTA might therefore instead be considered as a measure of microscopic traces left by a diet, instead of actual wear in the sense of quantitative tissue removal, until a better understanding of wear acquisition and development is reached on both the microscopic and macroscopic scales.

Some other theoretical considerations exist with respect to the effect of external abrasives on dental wear traces in ruminants. Schulz et al. (2010) suggested that a meso-distal wear gradient may still occur in ruminants, because during ingestion, the abrasives are washed out of the food bolus by saliva during the ingestive processing from the front to the rear of the oral cavity – a hypothesis that is difficult to test. In ruminants, the majority of masticatory particle size reduction occurs not during ingestion but during rumination (Trudell-Moore and White, 1983; McLeod and Minson, 1988). During ingestion, ruminants chew less consistently than during rumination (Dittmann et al., 2017), which might also lead to less pronounced wear effects due to ingestive versus rumination mastication. Prior to rumination, the ingested material is subject to peristaltic movements in the rumen liquid, which washes off external abrasives; those rumen contents from which material is recruited for regurgitation and rumination are therefore depleted of external abrasives compared with the ingested diet (Hatt et al., 2019). The regurgitate (also called ‘cud’) should therefore contain a lower amount of external abrasives. This washing mechanism may be less efficient with very small particles that are less prone to being washed off, which might explain the noticeable difference in the polishing effect between the two experimental diets with the smallest silica. However, how particle size determines the degree to which particles are washed off the ingested material in the rumen remains to be further explored. Washing may result in ruminant teeth losing tissue at a slower rate than in other ungulates, an issue to take into account when applying dental wear proxies on both macroscopic and microscopic scales in these animals. Wear traces of the diets used in the present study could be expected to be more distinct when investigated in non-ruminant animal models.

Conclusions

When analysing the molar teeth of sheep fed experimental diets with DMTA, there was no indication of an increase in microscopic traces along the molar row. However, DMT parameters indicated individual tooth differences, questioning the use of the M2 as the ideal tooth of reference as it is currently being used. Our results suggest that no particular molar needs to be prioritised. In fact, the use of all available teeth is common in palaeo-reconstructions. Regardless of tooth position, combining scans allowed for the strongest differentiation between dietary groups of different abrasiveness, emphasising that a higher number of measurements is advisable. Experimental diets containing abrasives of increasing size were distinguished by an increasing complexity, height and volume of the enamel surface textures, and when compared with the control diet, the smallest abrasives created a characteristic polishing effect. Whether microscopic traces on the enamel surface translate to a change in the tooth's overall volume is unknown. Further research is necessary to better understand whether indeed wear is created by processes that can be differentiated microscopically, and how this translates to the macroscopic scale.

Acknowledgements

This study was part of the AgroVet cooperation between Strickhof Eschikon and the Vetsuisse Faculty of the University of Zurich. We thank Anja Tschudin and Dietmar Ranz for support with pelleted diet formulation, and Hanspeter Renfer, Kaspar Luttiger, Röbi Sonderegger, Nadja Weber and Janine Hertaeg for support with animal husbandry. Steffen Lessle and Klaus Zwonarz are acknowledged for preparing the skulls.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.L.A., D.E.W., T.M.K., M.C.; Methodology: N.L.A., D.E.W., T.M.K.; Software: D.E.W., T.M.K.; Validation: N.L.A., T.M.K., J.-M.H.; Formal analysis: N.L.A., D.E.W.; Investigation: N.L.A., D.E.W., L.F.M., M.C.; Resources: T.M.K., M.C., J.-M.H.; Data curation: N.L.A., D.E.W.; Writing - original draft: N.L.E., D.E.W., M.C.; Writing - review & editing: N.L.A., L.F.M., T.M.K., M.C., J.-M.H.; Visualization: N.L.A.; Supervision: D.E.W., T.M.K., M.C., J.-M.H.; Project administration: D.E.W., T.M.K., M.C., J.-M.H.; Funding acquisition: N.L.A., T.M.K., M.C., J.-M.H.

Funding

This study was part of project 31003A_163300/1 funded by the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung and by a travel grant of the Graduate Campus of the University of Zurich to N.L.A. D.E.W's funding was provided by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC CoG grant agreement no. 681450) to Thomas Tütken.

Data availability

Data are available from the Dryad digital repository (Ackermans et al., 2020a): dryad.x95x69pdm

References

Ackermans, N. L., Martin, L. F., Hummel, J., Müller, D. W. H., Clauss, M. and Hatt, J.-M. (2019). Feeding selectivity for diet abrasiveness in sheep and goats. *Small Ruminant Res.* **175**, 160-164. doi:10.1016/j.smallrumres.2019.05.002

Ackermans, N. L., Winkler, D. E., Martin, L., Kaiser, T. M., Clauss, M. and Hatt, J.-M. (2020a). Dust and grit matter: abrasives of different size lead to opposing dental microwear textures in experimentally fed sheep (*Ovis aries*), v3, *Dryad*, Dataset. https://doi.org/10.5061/dryad.x95x69pdm

Ackermans, N. L., Martin, L. F., Codron, D., Kircher, P. R., Richter, H., Clauss, M. and Hatt, J.-M. (2020b). Confirmation of a wear-compensation mechanism in dental roots of ruminants. *Anat. Rec*. (in press).

Aiba, K., Miura, S. and Kubo, M. O. (2019). Dental microwear texture analysis in two ruminants, Japanese serow (*Capricornis crispus*) and sika deer (*Cervus nippon*), from central Japan. *Mamm. Study* **44**, 183-192. doi:10.3106/ms2018-0081

Baker, G., Jones, L. H. P. and Wardrop, I. D. (1959). Cause of wear in sheeps' teeth. *Nature* **184**, 1583-1584. doi:10.1038/1841583b0

Brent Jones, D. and Desantis, L. R. G. (2017). Dietary ecology of ungulates from the La Brea tar pits in southern California: a multi-proxy approach. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **466**, 110-127. doi:10.1016/j.palaeo.2016.11.019

Calandra, I., Schulz, E., Pinnow, M., Krohn, S. and Kaiser, T. M. (2012). Teasing apart the contributions of hard dietary items on 3D dental microtextures in primates. *J. Hum. Evol.* **63**, 85-98. doi:10.1016/j.jhevol.2012.05.001

Calandra, I., Pedergnana, A., Gneisinger, W. and Marreiros, J. (2019). Why should traceology learn from dental microwear, and vice-versa? *J. Archaeol. Sci.* **110**, 105012. doi:10.1016/j.jas.2019.105012

Clauss, M., Franz-Odendaal, T. A., Brasch, J., Castell, J. C. and Kaiser, T. (2007). Tooth wear in captive giraffes (*Giraffa camelopardalis*): mesowear analysis classifies free-ranging specimens as browsers but captive ones as grazers. *J. Zoo Wildl. Med.* **38**, 433-445. doi:10.1638/06-032.1

Clementz, M. T. (2012). New insight from old bones: stable isotope analysis of fossil mammals. *J. Mammal.* **93**, 368-380. doi:10.1644/11-MAMM-S-179.1

Daegling, D. J., Hua, L.-C. and Ungar, P. S. (2016). The role of food stiffness in dental microwear feature formation. *Arch. Oral Biol.* **71**, 16-23. doi:10.1016/j.archoralbio.2016.06.018

Dittmann, M. T., Kreuzer, M., Runge, U. and Clauss, M. (2017). Ingestive mastication in horses resembles rumination but not ingestive mastication in cattle and camels. *J. Exp. Zool. A* **327**, 98-109. doi:10.1002/jez.2075

Fandos, P., Orueta, J. F. and Aranda, Y. (1993). Tooth wear and Its relation to kind of food: the repercussion on age criteria in *Capra pyrenaica*. *Acta Thereologica* **38**, 93-102. doi:10.4098/AT.arch.93-8

Fortelius, M. and Solounias, N. (2000). Functional characterization of ungulate molars using the abrasion-attrition wear gradient: a new method for reconstructing paleodiets. *Am. Mus. Novit.* **3301**, 1-36. doi:10.1206/0003-0082(2000)301<0001:FCOUMU>2.0.CO;2

Gantt, D. G. (1979). Patterns of dental wear and the role of the canine in Cercopithecinae. *Am. J. Phys. Anthropol.* **51**, 353-359. doi:10.1002/ajpa.1330510306

Grine, F. E. (1986). Dental evidence for dietary differences in *Australopithecus* and *Paranthropu*s: a quantitative analysis of permanent molar microwear. *J. Hum. Evol.* **15**, 783-822. doi:10.1016/S0047-2484(86)80010-0

Hatt, J.-M., Codron, D., Müller, D. W. H., Ackermans, N. L., Martin, L. F., Kircher, P. R., Hummel, J. and Clauss, M. (2019). The rumen washes off abrasives before heavy-duty chewing in ruminants. *Mamm. Biol.* **97**, 104-111. doi:10.1016/j.mambio.2019.06.001

Hoffman, J. M., Fraser, D. and Clementz, M. T. (2015). Controlled feeding trials with ungulates: a new application of in vivo dental molding to assess the abrasive factors of microwear. *J. Exp. Biol.* **218**, 1538-1547. doi:10.1242/jeb.118406

Hua, L.-C., Brandt, E. T., Meullenet, J.-F., Zhou, Z.-R. and Ungar, P. S. (2015). An in vitro study of dental microwear formation using the BITE Master II chewing machine. *Am. J. Phys. Anthropol.* **158**, 769-775. doi:10.1002/ajpa.22823

Kaiser, T. M. and Fortelius, M. (2003). Differential mesowear in occluding upper and lower molars: opening mesowear analysis for lower molars and premolars in hypsodont horses. *J. Morphol.* **258**, 67-83. doi:10.1002/jmor.10125

Kaiser, T. M., Clauss, M. and Schulz-Kornas, E. (2016). A set of hypotheses on tribology of mammalian herbivore teeth. *Surf. Topogr. Metrol. Prop.* **4**, 014003. doi:10.1088/2051-672X/4/1/014003

Karme, A., Rannikko, J., Kallonen, A., Clauss, M. and Fortelius, M. (2016). Mechanical modelling of tooth wear. *J. R. Soc. Interface* **13**, 20160399. doi:10.1098/rsif.2016.0399

Kay, R. F. and Covert, H. H. (1983). True grit: a microwear experiment. *Am. J. Phys. Anthropol.* **61**, 33-38. doi:10.1002/ajpa.1330610104

Kropacheva, Y. E., Zykov, S. V., Smirnov, N. G. and Salimov, R. M. (2019). Dental microwear and mesowear of the *Microtus* voles molars before and after experimental feeding of owls. *Dokl Biol. Sci.* **486**, 79-82. doi:10.1134/S0012496619030049

Lucas, P. W., Omar, R., Al-Fadhalah, K., Almusallam, A. S., Henry, A. G., Michael, S., Thai, L. A., Watzke, J., Strait, D. S. and Atkins, A. G. (2013). Mechanisms and causes of wear in tooth enamel: implications for hominin diets. *J. R. Soc. Interface* **10**, 20120923. doi:10.1098/rsif.2012.0923

McLeod, M. N. and Minson, D. J. (1988). Large particle breakdown by cattle eating ryegrass and alfalfa. *J. Anim. Sci.* **66**, 992-999. doi:10.2527/jas1988.664992x

Merceron, G., Escarguel, G., Angibault, J.-M. and Verheyden-Tixier, H. (2010). Can dental microwear textures record inter-individual dietary variations? *PLoS ONE* **5**, e9542. doi:10.1371/journal.pone.0009542

Merceron, G., Hofman-Kamińska, E. and Kowalczyk, R. (2014). 3D dental microwear texture analysis of feeding habits of sympatric ruminants in the Białowieża Primeval Forest, Poland. *For. Ecol. Manag.* **328**, 262-269. doi:10.1016/j.foreco.2014.05.041

Merceron, G., Ramdarshan, A., Blondel, C., Boisserie, J.-R., Brunetiere, N., Francisco, A., Gautier, D., Milhet, X., Novello, A. and Pret, D. (2016). Untangling the environmental from the dietary: dust does not matter. *Proc. R. Soc. B* **283**, 20161032. doi:10.1098/rspb.2016.1032

Mihlbachler, M. C., Foy, M. and Beatty, B. L. (2019). Surface replication, fidelity and data loss in traditional dental microwear and dental microwear texture analysis. *Sci. Rep.* **9**, 1595. doi:10.1038/s41598-018-37682-5

Monson, T. A. and Hlusko, L. J. (2018). The evolution of dental eruption sequence in artiodactyls. *J. Mamm. Evol.* **25**, 15-26. doi:10.1007/s10914-016-9362-9

Pérez-Pérez, A., Espurz, V., de Castro, J. M. B., de Lumley, M. A. and Turbón, D. (2003). Non-occlusal dental microwear variability in a sample of Middle and Late Pleistocene human populations from Europe and the Near East. *J. Hum. Evol.* **44**, 497-513. doi:10.1016/S0047-2484(03)00030-7

Purnell, M., Seehausen, O. and Galis, F. (2012). Quantitative three-dimensional microtextural analyses of tooth wear as a tool for dietary discrimination in fishes. *J. R. Soc. Interface* **9**, 2225-2233. doi:10.1098/rsif.2012.0140

Ramdarshan, A., Blondel, C., Gautier, D., Surault, J. and Merceron, G. (2017). Overcoming sampling issues in dental tribology: insights from an experimentation on sheep. *Palaeontol. Electron.* **20**, 1-19. doi:10.26879/762

Romero, A., Galbany, J., De Juan, J. and Pérez-Pérez, A. (2012). Brief communication: short- and long-term in vivo human buccal–dental microwear turnover. *Am. J. Phys. Anthropol.* **148**, 467-472. doi:10.1002/ajpa.22054

Sanson, G. D., Kerr, S. and Read, J. (2017). Dietary exogenous and endogenous abrasives and tooth wear in African buffalo. *Biosurf. Biotribol.* **3**, 211-223. doi:10.1016/j.bsbt.2017.12.006

Schubert, B. W., Ungar, P. S. and DeSantis, L. R. G. (2010). Carnassial microwear and dietary behaviour in large carnivorans. *J. Zool.* **280**, 257-263. doi:10.1111/j.1469-7998.2009.00656.x

Schulz, E., Calandra, I. and Kaiser, T. M. (2010). Applying tribology to teeth of hoofed mammals. *Scanning* **32**, 162-182. doi:10.1002/sca.20181

Schulz, E., Calandra, I. and Kaiser, T. M. (2013a). Feeding ecology and chewing mechanics in hoofed mammals: 3D tribology of enamel wear. *Wear* **300**, 169-179. doi:10.1016/j.wear.2013.01.115

Schulz, E., Piotrowski, V., Clauss, M., Mau, M., Merceron, G. and Kaiser, T. M. (2013b). Dietary abrasiveness is associated with variability of microwear and dental surface texture in rabbits. *PLoS ONE* **8**, e56167. doi:10.1371/journal.pone.0056167

Scott, J. R. (2012). Dental microwear texture analysis of extant African Bovidae. *Mammalia* **76**, 157-174. doi:10.1515/mammalia-2011-0083

Scott, R. S., Ungar, P. S., Bergstrom, T. S., Brown, C. A., Childs, B. E., Teaford, M. F. and Walker, A. (2006). Dental microwear texture analysis: technical considerations. *J. Hum. Evol.* **51**, 339-349. doi:10.1016/j.jhevol.2006.04.006

Taylor, L. A., Kaiser, T. M., Schwitzer, C., Müller, D. W. H., Codron, D., Clauss, M. and Schulz, E. (2013). Detecting inter-cusp and inter-tooth wear patterns in Rhinocerotids. *PLoS ONE* **8**, e80921. doi:10.1371/journal.pone.0080921

Taylor, L. A., Müller, D. W. H., Schwitzer, C., Kaiser, T. M., Castell, J. C., Clauss, M. and Schulz-Kornas, E. (2016). Comparative analyses of tooth wear in free-ranging and captive wild equids. *Equine Vet. J.* **48**, 240-245. doi:10.1111/evj.12408

Teaford, M. F. and Oyen, O. J. (1989a). Differences in the rate of molar wear between monkeys raised on different diets. *J. Dent. Res.* **68**, 1513-1518. doi:10.1177/00220345890680110901

Teaford, M. F. and Oyen, O. J. (1989b). In vivo and in vitro turnover in dental microwear. *Am. J. Phys. Anthropol.* **80**, 447-460. doi:10.1002/ajpa.1330800405

Teaford, M. F., Ungar, P. S., Taylor, A. B., Ross, C. F. and Vinyard, C. J. (2017). *In vivo* rates of dental microwear formation in laboratory primates fed different food items. *Biosurf. Biotribol.* **3**, 166. doi:10.1016/j.bsbt.2017.11.005

Trudell-Moore, J. and White, R. G. (1983). Physical breakdown of food during eating and rumination in reindeer. *Acta Zool. Fenn.* **175**, 47-49.

Ungar, P. S., Merceron, G. and Scott, R. S. (2007). Dental microwear texture analysis of Varswater bovids and early Pliocene paleoenvironments of Langebaanweg, Western Cape Province, South Africa. *J. Mamm. Evol.* **14**, 163-181. doi:10.1007/s10914-007-9050-x

Walker, A., Hoeck, H. N. and Perez, L. (1978). Microwear of mammalian teeth as an indicator of diet. *Science* **201**, 908-910. doi:10.1126/science.684415

Wilcox, R. R. (2012). *Introduction to Robust Estimation and Hypothesis Testing*. Elsevier.

Winkler, D. E., Schulz-Kornas, E., Kaiser, T. M., De Cuyper, A., Clauss, M. and Tütken, T. (2019a). Forage silica and water content control dental surface texture in guinea pigs and provide implications for dietary reconstruction. *Proc. Natl Acad. Sci. USA* **116**, 1325-1330. doi:10.1073/pnas.1814081116

Winkler, D. E., Schulz-Kornas, E., Kaiser, T. M. and Tütken, T. (2019b). Dental microwear texture reflects dietary tendencies in extant Lepidosauria despite their limited use of oral food processing. *Proc. R. Soc. B* **286**, 20190544. doi:10.1098/rspb.2019.0544